LESSON 2:
LABORATORY ORGANIZATION FOR PLANT TISSUE CULTURE (IN VITRO METHODS)

Objective

This lesson deals with the basic laboratory set- up that is required to initiate plant tissue culture work. The laboratory set-up for tissue culture depends on the nature of the research undertaken and the availability of funds. For a standard tissue culture laboratory, following minimum facilities are necessary:

i. Washing & storage facilities

ii. Media preparation, sterilization & storage room

iii. Transfer area for aseptic manipulations

iv. Culture rooms or incubators for maintenance of cultures under controlled conditions of temperature, light & humidity

v. Observation or data collection area

Let us observe each area of the Laboratory:

The following diagram (Fig.1) will give you an idea as to how a plant tissue culture laboratory should be organized. The diagram is followed by details on each area of the laboratory.

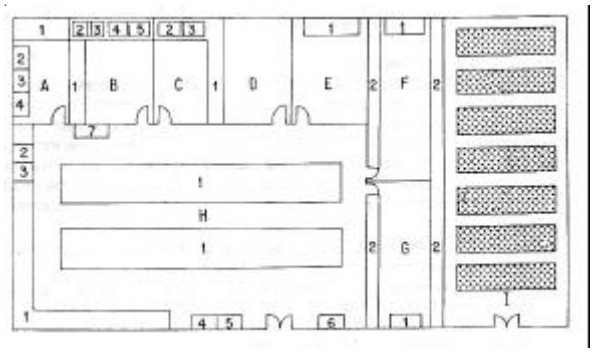

Fig. 1. Layout for organisation of a tissue Culture laboratory. A: Media preparation and sterillsation room with lab bench (1), space for autoclave (2), water bath (3), heat-dry sterilisation unit (4); B: Washing area with lab bench (1), double-basin, lead-lined sink with provision for running water taps (2), space for detergent baths, brushes etc. (3), draining-boards/racks (4), drying ovens (5); C: Dark room with lab bench (1), refrigerator and deep-freeze space (2), double-basin. Lead-lined sink (3); D: Storage room for chemicals and glassware; E: Transfer room fitted with overhead UV and HEPA filter ventilation unit, laminar air-flow hood (1) for aseptic manipulations; F-G: Temperature-controlled culture room with shaking machines installed (1), culture racks (2) fitted with light-adjustable fluorescent tubes; H: Main laboratory with lab bench and tables (1) for microscopy work, data collection etc., centrifuge (2), pH meter (3), hot plate / stirrers (4), balances (5), space for buckets and used labware (6), double-distillation unit (7), within close access to A and B; l: Transplantation area with controls for light, temperature and humidity (modified from Torres 1989)

Washing & Storage Facilities: (See Appendix 1)

New glassware is always washed using detergents especially designed for the purpose to remove all traces of acids, etc. Finally, the glassware is rinsed in tap water and then in distilled water. For this, sufficient area is required to accommodate large sinks (acid-lined), hot and cold running water, draining boards/racks and sufficient distilled/demineralized water. Space should also be available to set up drying ovens; for storage of washed and dried labware; the washing area should have dustproof cupboards, etc.

In tissue culture, glassware should be resistant to heat. Cleaning of glasswares is done by soaking it in sodium dichromate-sulphuric acid (conc.) for 4 hrs. and then washing under tap water. The glassware is then soaked in a detergent solution for 16 hrs, then rinsed first in tap water followed by a second rinse in distilled water. Glassware is then dried in oven at high temperature.

Instead of glasswares, a wide range of presterilised polystyrene culture containers (plastic labwares) are now available which can be disposed off after use. Reusable plastic labware is also available now. These can be washed with a mild detergent and rinsed with tap and distilled water.

Washed and dried labware is finally stored in a closed cupboard (dust free).

Media Preparation Room

This room should have sufficient space for storage of chemicals, labware, culture vessels, pH meters, balances, water baths, burners etc. A microwave oven, autoclave, or domestic pressure cooker for sterilizing media, culture vessels and instruments are appliances most needed for media preparation (see Appendix 2 and 3).

Transfer Area:

The simplest type of transfer area is an enclosed plastic box which can be sterilized with a UV light and by cleaning the floor surface with 70% ethyl alcohol. A small wooden hood may also be used for tissue culture work.

When a large number of cultures or transfers are being manipulated, large equipment is required and the most desirable arrangement is a dust – free room equipped with an overhead UV and positive pressure ventilation unit. The ventilation should possess a high-efficiency particulate air (HEPA) filter. It has been observed that a 0.3um HEPA filter shows almost 100 percent efficiency in not all the bacteria to pass through. All the surfaces in the room can be thoroughly cleansed and disinfected regularly. The room should be air-conditioned.

Another type of transfer area used currently in most laboratories is a 'laminar airflow cabinet'. A small motor blows air into the unit first through a coarse filter, where large dust particles are separated, and then, passes through a 0.3um HEPA filter. The

air is directed either downward (vertical flow unit) or outward (horizontal flow unit) over the working surface. The air coming out of the fine filter is ultraclean (free from fungal or bacterial contaminant) and its velocity (27+ 3m/min) adequately prevents the microcontamination of the working area by a worker sitting in front of the cabinet. Various other contaminants (e.g. hair, salts, etc.) are also blown away by the ultraclean airflow. In this way, an aseptic environment is maintained when the cabinet is switched on. Laminar airflow cabinets are commercially available in various sizes and shapes. The advantage of using such cabinets is that the flow of air does not hamper the use of a spirit lamp or Bunsen burner and each cabinet occupies a relatively small space within an ordinary lab. At places where atmospheric dust is very high, it is advisable to keep the airflow cabinet in a culture room fitted with double doors to prolong the life of filter. An important precaution is that a laminar airflow cabinet should never face a window or door that is frequently used (see Appendix 4).

Culture Room

All types of plant tissues are incubated under conditions of well-controlled temperature, humidity, illumination and air circulation. Usually, air-conditioners and heaters are used to maintain the temperature around 25± 2° C. Cultures are generally grown in diffused light. Other requirements are a humidity range of 20-98%, controllable to ±3% and uniform forced–air ventilation. Culture room should have enough shelves illuminated by a set of fluorescent tubes for storing cultures. All the cultures should be labelled, giving details of the experiment (name of the plant, explant, medium, date of culture and other information) to ensure identity and for recording monitored results. Shakers with controlled temperature and light are also installed in a culture room.

Incubators, large plant growth chambers, are readily available in the market. They occupy less space and have the range of control and flexibility desirable for growth of tissues under in vitro conditions (see Appendix 2).

Observation or Data Collection Area

The growth and development of tissues cultured in vitro is generally monitored by observing cultures at regular intervals, in the culture room. Plants regenerated from in vitro tissue cultures are transplanted to soil in pots. The potted plants are ultimately transferred to green houses or growth cabinets and maintained for further observations under controlled conditions of light, temperature and humidity.

Appendix 1

Additional Guidelines for Washing and Handling of Glassware

General

1. Reusable glassware for tissue culture should be emptied immediately after use and soaked. Never allow media or agar to dry on the glassware.
2. Segregate all glassware containing corrosive chemicals or fixatives from the rest of the tissue culture glassware.
3. All glassware contaminated or coming into contact with micro-organisms should be decontaminated before washing.
4. Remove labels and marking ink before washing. Marking ink may be wiped out by abrasive cleanser or acetone and immediate rinsing.
5 Melted agar in the culture vessels should be poured into a collecting sieve and discarded.
6 Rubber-lined screw caps should be soaked only in distilled water. Never soak caps in detergent or soap solution.
7. Glassware for growing cells should be acid-cleaned if proteinaceous deposits are not removed by conventional washing.
8. Silicone-treated glassware should be permanently labelled and placed separately from the rest of the glassware.

Glass-washing

1. Tissue culture glassware should be soaked in 5% detergent solution for a minimum of 1 hr.
2. The automatic washer is used only as a rinsing unit and no detergent is added to the washer. Glassware should be treated with detergent prior to its transfer into the washing unit.
3. The automatic washer is programmed for no less than six tap water rinses and a 1 min distilled water rinse.
4. The glassware that does not fit into an automatic unit is rinsed by hand. Hand-rinsing requires a minimum of six thorough tap water rinses and four rinses in quality water.
5. If an automatic dish-washing facility is not available, a simple washing machine with brushes of different sizes may be used for manual washing.

Acid-cleaning

1. Personnel concerned should wear a full-face mask and acid-resistant apron and gloves.
2. Handling of acid and processing of glassware in the acid should be done in a fume hood.
3 Never add water to acid. To dilute acid, add acid slowly to water while stirring.
4. After acid-cleaning, if the solution in the acid bath becomes dark coloured, it should be discarded.

Appendix 2

General Characteristics of Commercially Available Incubators or Growth Chambers

1. Temperature range, 2- 40°C.
2. Temperature control, ± 0.5°C.
3. Safety high- and low-temperature limits.
4. Continuous temperature recorder.
5. Twenty-four-hour temperature and light programming.
6. Adjustable fluorescent lighting up to 10,000 Ix.
7. Relative humidity range, 20-98%.
8. Relative humidity control ±3%.
9. Uniform forced-air distribution.
10. Capacity upto 0.7 m3 of 0.5 m^2 shelf space.

Appendix 3

Equipments Commonly Used in a Laboratory for Many Tissue Culture Techniques

Preparation of Media

1. Gas, water, and electricity supplies.
2. Provision for compressed air and vacuum lines.
3. Water heater or small stove.
4. Hot plate with magnetic stirrer.
5. Glass or stainless steel containers for heating and dissolving media.
6. Autoclave or pressure-steam steriliser.
7. pH meter.
8. Centigram balances.
9. Graduated measuring cylinders, flasks, beakers, petri dishes and pipettes with teats.
10. Culture tubes, bottles and other glassware with suitable closures (such as cotton plugs, aluminium foil, plastic film, or metal caps).
11. Small transfer instruments such as spatulas, scalpels, forceps, or dissecting needles.
12. Hot-air oven for rapid heating of media and agar mixtures (microwave oven will also do rapid thawing of frozen items).
13. Water de-ioniser, distilled or double-distilled water units.
14. Filter membranes with holders and hypodermic syringes to filter-sterilise solutions.
15. Chemicals for preparing culture media or commercially available powdered culture media, growth hormones and other organic constituents.
16. Detergents, disinfectants.
17. Pipette washers (acid proof).
18. Drying and draining racks.
19. Storage space for chemicals, glassware, nutrient media, sterile water and other items.

Isolation of Cultures

1. Laminar airflow cabinet.
2. Spirit lamp or bunsen burner in the inoculation cabinet.
3. Stereo-microscope.
4. Ethyl alcohol (70%) for sterilisation and flaming of small metal instruments.
5. Tiles or glass plates for use during sterile cutting.
6. Hypochlorite solution for sterilisation of plant material.

General Equipment

1. Refrigerator, deep-freeze.
2. Automatic dish-washer.
3. Glass atomiser.
4. Dispensing devices (e.g., wire-mesh baskets, trolleys witb trays and metal racks for holding test-tubes or culture vials in the autoclave).
5. Acid proof baths for cleansing glassware.
6. Microscopes (e.g., compound, inverted) with microphotographic equipment. .
7. Markers, labels and vitafilm (or similar material for wrapping culture vessels, glassware, tiles and other labware).

Culture Room

1. Temperature control (17-27°C).
2. Electricity supply essential for lighting, cooling and heating.
3. Shelves for culture racks.
4. Fluorescent tubes for lighting.
5. Timer for regulating day-length.
6. Racks for culture vials.
7. Rotary shaker.
8. Observations table.

Transplantation Facility

A small area where high humidity, light and temperature can be controlled in the form of a greenhouse or a small room.

Appendix 4

Operation of Laminar Airflow Hood

1. Switch on light and blower motor 15 min before making transfers.
2. Check to make sure that no paper or other object blocks the air intake at the bottom of the unit and that the sterile air coming out of the filter flows uniformly in the hood.
3. Use 70% ethanol to wipe the working surface of the hood.
4. Place only sterile instruments, appliances and culture tubes or vessels inside the hood.
5. During flame sterilisation of the small instruments keep spirit lamp or bunsen burner at a sufficient distance from the bottle containing alcohol to avoid fire.
6. After using the hood, rewipe its surface with ethanol, remove all appliances, dishes, or containers, and switch off the light and blower motor. Cover the hood with an airtight screen.

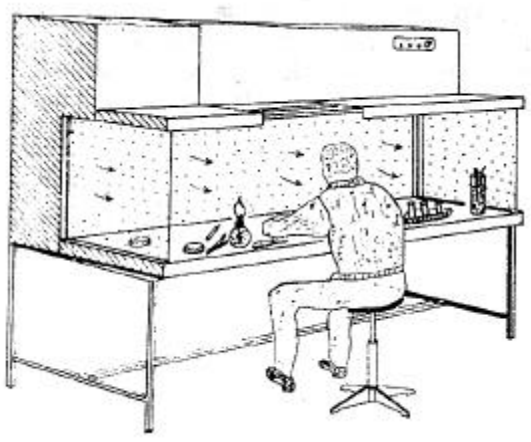

Fig. 2. Laminar Air Flow Hood

Conclusion

Plant tissue culture laboratory is organized in such a way that there should be specific area for each operation. This is done mainly to avoid the problem of contamination (due to fungus, microbes etc.) which might ruin all the cultures in no time. Moreover, growth of callus and cultures have specific requirements of light , temperature, etc. which have to be met in a proper way. Therefore, a plant tissue culture laboratory has to be planned according to the requirements of the cultures.

Questions

1. What are the requirements for establishing a tissue culture laboratory?
2. How does a Laminar air flow station operate?
3. What all equipments do you need to run a plant tissue culture laboratory?

Note